Dispatches

Behavioral Neuroscience: Learning to Suckle with Signature Odor

A new study in mice reveals that an apparently innate behavior, suckling, is triggered not by a classical pheromone but by the pup learning the complex signature odor of its mother.

Jennifer J. Bussell[1] and Leslie B. Vosshall[1,2,*]

As chemical keys that unlock specific responses from other members of a species, pheromones hold particular appeal. Pheromones were first identified in the silk moth, *Bombyx mori*, and the female moth sex pheromone, bombykol, still serves as one of the best studied examples [1]. Upon sensing minute quantities of bombykol in the air, a male moth will immediately orient towards it and attempt to find and mate with the female. Classically, a pheromone is defined as a single molecule or a defined ratio of multiple molecules in a mixture and by the receiver not needing prior experience to produce the appropriate response on first exposure [2]. Even though many pheromones and the behaviors they trigger have been identified, particularly in insects, molecules that fit the definition of a pheromone have not yet been conclusively identified in humans. Moreover, some have argued that the strict definition of a pheromone may not be applicable to cues in mammals, with their more complicated olfactory systems and larger brains [3]. The term 'signature mixture' has been proposed to describe olfactory cues that are more variable across individuals than pheromones and that must be learned by conspecifics to trigger behavior [4]. In a paper in this issue of *Current Biology*, Logan *et al.* [5] demonstrate that, in the mouse, suckling behavior is cued by maternal odor that the mouse pup learns either in the womb or immediately after birth. The complex maternal odor learned by the mouse pup is better described as a signature mixture than a classical pheromone.

Mammalian suckling seems to be innate, as most newborns suckle successfully at their first attempt. Previous work in European rabbits (*Oryctolagus cuniculus*) identified a suckling cue in milk, 2-methylbut-2-enal, that fits the classical definition of a pheromone: it is species-specific, is monomolecular, and releases an immediate behavior [6]. However, mothers of this species show an unusual behavior: they protect their pups from predators by avoiding them most of the time and are only available to suckle for about four minutes per day [7]. The context of rabbit suckling may, therefore, require particularly robust cues that are not common to all mammals.

Logan *et al.* [5] set out to identify suckling cues in the mouse. Mice are quite helpless at birth — blind, with limited mobility, and a need to stay constantly close to the mother to keep warm (Figure 1A). Gestation and birth expose pups to a variety of maternal fluids, all of which could provide chemosensory cues for suckling. As each pup in a litter is born, the mother ruptures the amniotic sac and licks the pup clean, which stimulates its breathing. After completion of delivery, she licks clean her own belly, including the nipples, and then allows the pups to suckle. Thus the pups are exposed to a blend of amniotic fluid, saliva, and milk within minutes of birth (Figure 1B). Logan *et al.* [5] developed a simple and elegant assay for initial suckling behavior in which the mother's nipple is washed to remove any possible olfactory cues. Mouse pups do not suckle a washed nipple even if they are placed directly on it and receive potential tactile or heat cues.

Making the Case for a Signature Odor That Triggers Suckling

With the mother's washed nipple as a blank canvas, the authors painted on potentially bioactive fluids and found that those present at around the time of birth — amniotic fluid, maternal saliva, or milk — restored suckling. They next manipulated the mother's cleansing of the pups by delivering them by Caesarian section. In this case, only amniotic fluid, to which the pups had been exposed in the womb, triggered suckling when presented on the washed nipple. To gather more evidence that perinatal experience was crucial to teach pups about suckling, the authors themselves painted the pups with a wide range of substances, mimicking the mother's licking. Astonishingly, every substance tested could restore suckling behavior, including birth-irrelevant substances such as virgin saliva and vanillin. When pups were exposed to a blend of synthetic odorants shortly after birth, only the blend and not the individual odorant triggered suckling (Figure 1C).

The authors next embarked on a biochemical journey to find the substance or substances in amniotic fluid that trigger suckling behavior. They found that no single biochemical fraction had activity, but blends did. This implies either that there is a suckling pheromone composed of multiple components, or more radically, that the odor cue for suckling is learned by perinatal exposure. To test this latter idea, the authors added vanilla to amniotic fluid, which eliminated suckling behavior. This suggests the pups have 'imprinted' to the complex odor mixture of amniotic fluid, and if that odor is altered, it no longer seems familiar and fails to trigger the behavior.

As the ultimate test of the hypothesis that perinatal odor experience can train pups to suckle, the authors manipulated the maternal diet, scenting the amniotic fluid with garlic or vanilla by offering the mother garlic water or vanilla water during pregnancy. Remarkably, pups would then only suckle on the nipple reminiscent of their prior scent experience (Figure 1D). Looking at the complete evidence they gather, Logan *et al.* [5] propose that suckling in mice is triggered by the signature odor of the mother, which is learned

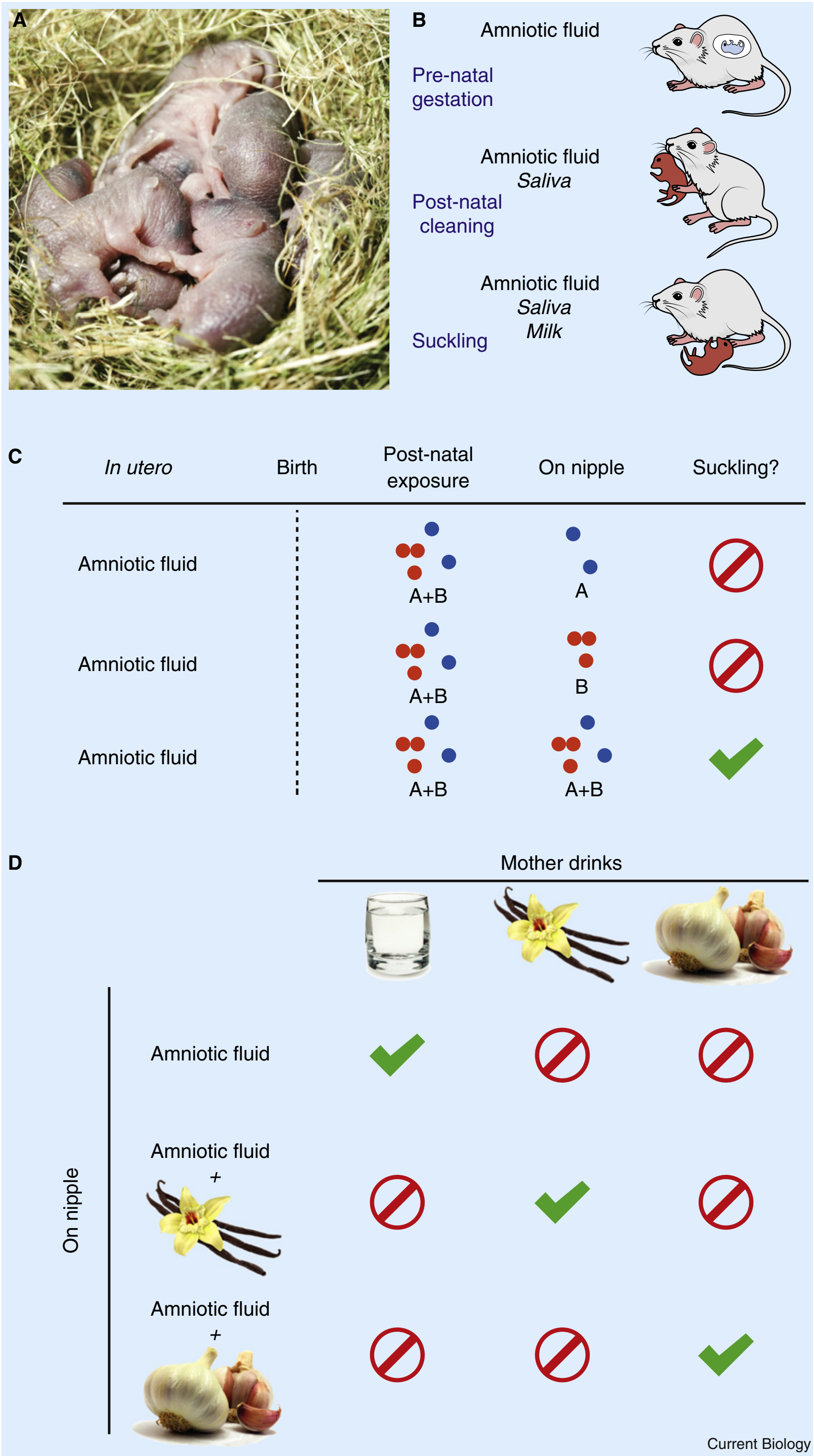

Figure 1. Chemical cues and training that can elicit pup suckling.

(A) Mouse pups are blind at birth and have limited mobility. (B) Pups are exposed to a variety of maternal substances from gestation to suckling. (C) Mouse pups birthed by Caesarian section and thus not exposed to maternal substances will suckle in response to the odor blend (A+B) they were exposed to post-natally but not components of the blend (A or B). (D) Pups suckle only in response to amniotic fluid chemically matched to that which they experienced *in utero*. (Photos: Fotolia.com: mouse pups (© gigi1807); water (© Sergey Yarochkin); vanilla (© rimglow); garlic (© Schlierner)).

in the perinatal period and is distinct from a pheromone with intrinsic bioactivity.

The failure to find a murine suckling pheromone of course does not guarantee that such a substance does not exist. Pheromones can be labile, difficult to purify, or blends of two or more molecules that are not easily reconstituted. That said, Logan and co-workers [5] provide a number of compelling lines of evidence that suckling in mice can be released by virtually any odor to which the pups are trained in the perinatal period (Figure 1C).

The details and the critical period of how mouse pups learn maternal signature odors will be interesting to explore. The authors show that both late-stage developing embryonic and neonatal pup olfactory sensory neurons respond to amniotic fluid and other odors. Is the neonate brain hardwired to suckle once it senses the odor it experienced in the womb? Or is the mother's postnatal licking the cue to bind odor to suckling behavior? Teasing apart how the brain associates odors from particular experiences, which may not be in themselves rewarding, with later behavior could provide general insight into innate behaviors.

All mammals possess the innate drive to suckle at the mother's breast immediately after birth, but humans are unique among mammals in being able to offer bottled formula alternatives to natural suckling. In fact, many human mothers experience difficulty even at the initial breastfeeding [8], and it could be that the human suckling cue is lost in modern clinical hygienic birth practices. Further insights into how the neonatal mouse learns the mother's signature odor as well as research into whether human infants need a similar cue might even benefit breastfeeding women.

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[1]Laboratory of Neurogenetics and Behavior, [2]Howard Hughes Medical Institute, The Rockefeller University, 1230 York Avenue, Box 63, New York, NY 10065, USA.
*E-mail: leslie.vosshall@rockefeller.edu

http://dx.doi.org/10.1016/j.cub.2012.09.034

Evolution: Drift Will Tear Us Apart

That the widely scattered geographical distribution of some animals could be due to continental drift is a neat idea. Now, cave animals provide evidence for extreme long-term persistence on continents drifting apart.

Florian Maderspacher

It was the 6th of January 1912, epiphany in Christian calendars, when a 31-year-old man stepped up on the stage to address the German Geological Society's annual assembly in Frankfurt. He was a geological nobody. Instead of rocks, he had studied clouds and the ice of Greenland, and what he had to say sounded ludicrous: "New Ideas on the Formation of Large-Scale Structures of the Earth's Crust". Why new ideas? Everyone knew that the continents had always been where they were in 1912. Yes, sea levels had risen and fallen, mountains emerged; and what about the animals whose identical fossils had been found on either side of the Atlantic? Well, they obviously had crossed on a now submerged land bridge. The idea that the young meteorologist by the name of Alfred Wegener put forward was that the continents had drifted into their current place from the break-up of a large landmass that once comprised all current continents. To him, this explained everything — from the shapes of continents fitting together like puzzle pieces to the similar species found on either side of uncrossable oceans. Yet, the audience ridiculed his idea as a 'fever fantasy', and it wasn't widely accepted until decades after Wegener's untimely death in 1929. A hundred years on, Wegener doesn't need our support anymore. But if he did, the phenomenon of cave animals becoming separated by continents drifting apart that Maria M. Bauzà-Ribot and colleagues [1] describe in this issue of *Current Biology* would surely please him. Think of it as a birthday gift to his theory.

Bauzà-Ribot and her team [1] investigated the phylogeny of a small family of cave-dwelling amphipods. Amphipods are a fairly large group of crustaceans (around 7,000 species), but to describe them as 'inconspicuous' would be a massive understatement — let's just say they look like small shrimp. Amphipods have a penchant for living in caves: more than ten percent of amphipods, in Europe nearly half, are stygobionts [2], meaning they dwell in subterranean waters (Styx was the mythological underworld river ancient Greeks had to cross to get to 'the other side', and coincidentally also the name of a 70s prog-rock band that is itself an example of long-term persistence).

The particular family they studied is the Metacrangonyctidae (the cumbersome name befits their cryptic nature and I promise this is the only time I will spell it out here), which comprises two genera of about 35 species, all of which live in caves. No disrespect, but these are your run-of-the-mill stygobionts: blind, pale, with long antennae. What is, however, noteworthy is where their caves are located, namely on opposite sides of the Atlantic [3]: they inhabit caves in Morocco, Mallorca, Elba and Fuerteventura, as well as on the Caribbean island of Hispaniola (Figure 1).

Bauzà-Ribot and colleagues [1] generated a phylogeny of 16 species in their family based on entire mitochondrial genomes and some nuclear data. Strangely, all of the island species form a monophyletic group, meaning they all descended from a common ancestor, despite their very scattered distribution. Even the one species living on the Canary island of Fuerteventura is more closely related to species on islands thousands of miles away than to species on the relatively nearby Moroccan mainland. That such related species should live in caves separated by a vast ocean is weird, because it is at odds with the usually fairly narrow geographic ranges of cave animals, yet the same pattern is seen with a few other kinds of cave crustaceans [4,5]. How can this be?

Vicariance Vindicated

The distribution of any biological entity — be it a species, a group of species, or a population — can be

Figure 1. Spelunking for stygobionts.
Brackish waters in Cova de Cala Varques, located on the eastern coast of Mallorca. This body of brackish waters, separated from the underlying sea-water by a major halocline, is a typical habitat for the amphipod *Metacrangonyx longipes* (Photo: Tomeu Cañellas).